FURTHER STUDIES OF SOLUTION TENSION AND TOXICITY IN LIPOLYSIS[1]

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This paper is really a continuation of an earlier one[2] which supplied data showing that the toxicity of certain salts in lipolysis does not vary inversely as the decomposition tension of those salts. As my conclusion disagrees with that of MATHEWS,[3] MCGUIGAN,[4] and CALDWELL,[5] and as BERG and GIES[6] found that the nature of the zymolyte is an important factor in peptolysis and tryptolysis, it seemed desirable to determine whether the same general relation found by me for the toxicity of several nitrates in the lipolysis of ethyl butyrate would also hold in the case of some other ester. Ethyl acetate was selected as the zymolyte because its saponification is a monomolecular reaction, because acetic acid is a close homologue of butyric acid, and because LOEVENHART and PEIRCE[7] found that the hydrolysis of ethyl acetate is inhibited to a greater extent by sodium fluorid than that of ethyl butyrate. They also expressed the view that the inhibiting effect of sodium fluorid is dependent upon the acid radical from which the ester is derived, while the alcohol arising in the saponification is a comparatively indifferent factor.

Since the same general procedure has been followed here as in the preceding investigation, many of the details given before will be omitted

1 From the laboratories of the New York Botanical Garden.

2 POND, RAYMOND H., Solution tension and toxicity in lipolysis. Amer. Jour. Physiology 19:258–283. 1907. Contributions from the New York Botanical Garden 94.

3 MATHEWS, A. P., The relation between solution tension, atomic volume, and the physiological action of the elements. Amer. Jour. Physiology 10:290–323. 1904.

4 MCGUIGAN, HUGH M., The relation between the decomposition tension of salts and their antifermentative properties. Amer. Jour. Physiology 10:444–451. 1904.

5 CALDWELL, J. S., The effects of toxic agents upon the action of bromelin. BOT. GAZETTE 39:409–419. 1905.

6 BERG, W. N., and GIES, WM. J., Studies of the effects of ions on catalysis, with particular reference to peptolysis and tryptolysis. Jour. Biol. Chem. 2:489–546. 1907.

7 LOEVENHART, A. S., and PEIRCE, GEO., The inhibiting effect of sodium fluorid on the action of lipase. Jour. Biol. Chem. 2:379–413. 1907.

here. Most of the solutions used were made up from the same stock solutions and all were upon the same basis of molecular weight. The lipase used was taken from the same sample. Professor GIES has been my counselor in this work as in the other, and the same acknowledgments are due the New York Botanical Garden for the exceptionally favorable facilities afforded.

METHOD

When ethyl acetate is decomposed by the action of lipase, the products are supposed to be the same as in any case of its saponification, namely, acetic acid and ethyl alcohol. The amount of acid liberated from initially neutral acetate under given conditions is an index of the amount of enzyme activity under those conditions, and can be expressed in cubic centimeters of KOH solution required for neutralization. Since boiling destroys the activity of the enzyme, and since the acetate does not become appreciably acid in such a boiled solution of the enzyme during an incubation of 5 hours at 40° C., a boiled solution of the enzyme can be used as a control. The difference then in acidity of a boiled and of an unboiled solution of the enzyme of equal concentration, to which the same amount of ester has been added, expresses the amount of enzyme activity under the conditions imposed. Six vials were used for each concentration of the toxic salt. To each vial were added 2^{cc} of the toxic salt solution of a certain concentration, say $m/32$. Then to each of three of the vials were added 2^{cc} of the unboiled, filtered enzyme solution having a given concentration, say 0.10 per cent. To each of the other three vials were then added 2^{cc} of a portion of the same enzyme solution which had been boiled. Then to each of the six vials was added 0.10^{cc} of neutral ethyl acetate free from alcohol. The stoppered vials were placed in an incubator for 5 hours at 40°C. After incubation each vial was titrated with $m/40$ KOH, using phenolphthalein as an indicator. The difference between the boiled and unboiled solutions showed the amount of enzyme activity for $m/64$ of the salt used with a concentration of the enzyme of about 0.05 per cent. Filtering the enzyme solution makes the exact concentration uncertain. All of the salts used were nitrates except that of mercury, which was the bichlorid.

Since the acetate has the lower boiling-point and decomposes more rapidly in the presence of water, one would expect that it would

be more readily saponified by the lipase. A 0.0125 per cent. solution of the enzyme yielded from 0.10^{cc} of butyrate enough acid to require 0.20^{cc} of $m/20$ KOH for neutralization. The same concentration of enzyme acting for one hour longer at the same temperature on the same amount of acetate gave the figure 0.06^{cc} of $m/40$ KOH. A stronger solution of the enzyme (0.0250 per cent.) acting on the acetate gave 0.08^{cc} of $m/40$ KOH. From this one might think that the butyrate is the less stable in the presence of the enzyme, but it may be that the products of saponification are more inhibiting in the one case than in the other. The concentration of the enzyme in most of

TABLE I: RELATIVE TOXICITY WITH SAME CONCENTRATION OF ENZYME

Potassium and sodium in contemporaneous test

POTASSIUM

m	Control[8]	Enzyme[8]	Increase[8]
Water[8]........	0.07	0.25	0.18
32............	0.06	0.20	0.14
16............	0.06	0.19	0.13
8............	0.06	0.16	0.10
4............	0.06	0.13	0.07
2............	0.06	0.13	0.07
1............	0.06	0.10	0.04

SODIUM

32............	0.07	0.20	0.13
16............	0.07	0.17	0.10
8............	0.07	0.15	0.08
4............	0.07	0.13	0.06
2............	0.07	0.10	0.03
1............	0.07	0.10	0.03

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

[8] In the column headed "*m*" are given the various concentrations of the toxic salt in fractions of molecular strength. In this column the word "Water" means that 2^{cc} of water were used instead of the toxic solution in order to ascertain the uninhibited activity of the enzyme in 0.05 per cent. strength. In the column headed "Control" are given the figures for the final acidity (cc. $m/40$ KOH) of the boiled preparations containing the toxic salt in the concentration indicated by the corresponding figure in the column headed "*m*." The column headed "Enzyme" shows the final acidity of the unboiled preparations corresponding to the controls. The column headed "Increase" shows the difference in acidity between the controls and the unboiled preparations and expresses the amount of saponification allowed by the concentration of the toxic solution indicated by the corresponding figures in the column headed "*m*."

the tests was 0.05 per cent., that is, the filtrate obtained from the solution of 0.20^{gm} of the enzyme powder in 200^{cc} of water, filtered and then diluted one-half by the addition of an equal volume of the toxic solution. During 5 hours' incubation at 40° C., 0.1^{cc} of neutral acetate in 4^{cc} of water remains neutral.

In this test, as in several that follow, it was observed that while there was sediment in each of the vials containing boiled enzyme, the vials containing the unboiled solutions were at the close of the incubation entirely clear and free from sediment. There is no doubt therefore that partial inhibition of the enzyme is possible and actually occurs in the absence of any precipitation whatever. In this table we note that the uninhibited activity of the enzyme is expressed by 0.18^{cc}. The most concentrated solutions of the salts reduce this to 0.04 for potassium and to 0.03 for sodium. The amount of inhibition is therefore very considerable, and yet there was no precipitation even after an incubation period of 5 hours at 40° C. In this test the nitrates of sodium and of potassium are certainly to be regarded as equitoxic.

TABLE II: *Potassium, sodium, and lithium in contemporaneous test*

m	Control	Enzyme	Increase
POTASSIUM			
Water.........	0.10	0.25	0.15
8.............	0.10	0.15	0.05
4.............	0.10	0.13	0.03
1.............	0.10	0.13	0.03
SODIUM			
8.............	0.10	0.20	0.10
4.............	0.08	0.16	0.08
2.............	0.07	0.15	0.08
1.............	0.07	0.12	0.05
LITHIUM			
8.............	0.08	0.17	0.09
4.............	0.09	0.15	0.06
2.............	0.09	0.13	0.04

Enzyme 0.05 per cent. Incubation 4 hours at 45° C.

So far as this particular test is concerned, it can hardly be said that the salts are equitoxic. On the other hand, it cannot be positively

concluded which of the three is the most toxic. Comparison of the individuals of the series leads to nothing decisive. The next table shows that fluctuating differences occur.

TABLE III

POTASSIUM

m	Control	Enzyme	Increase
Water	0.09	0.23	0.14
8	0.10	0.20	0.10
4	0.09	0.18	0.09
2	0.09	0.16	0.07
1	0.09	0.12	0.03
SODIUM			
8	0.07	0.20	0.13
4	0.08	0.18	0.10
2	0.08	0.15	0.07
1	0.08	0.13	0.05
LITHIUM			
8	0.10	0.17	0.07
4	0.09	0.16	0.07
2	0.09	0.13	0.04

Enzyme 0.05 per cent. Incubation 5 hours at 45 C.

There seems to be some indication that lithium is more toxic than sodium or potassium, but the difference is too insignificant for a conclusion. In all the vials the unboiled solutions were free from sediment. Since the salt solutions themselves are neutral in all the concentrations tried, the column showing acidity of the reagent has been omitted. The titration with the $m/40$ KOH instead of the $m/20$ KOH used in the previous investigation with ethyl butyrate perhaps brings out greater variations. The acidity of the control is usually with each of these salts about the same in all concentrations and just about what the plain enzyme solution itself is. This confirms the observation made in the work with ethyl butyrate that there is no positive sign of any chemical action between the substance of the enzyme solution and that of the toxic solution.

In this test the two salts are neutral in all concentrations tried. One drop of the $m/40$ KOH gives a deep purple with 4^{cc} of the salt solution in any of the concentrations used in the test. The unboiled preparations were all free from sediment after incubation. Total inhi-

TABLE IV: *Barium and magnesium in contemporaneous test*

m	Control	Enzyme	Increase
BARIUM			
Water	0.10	0.22	0.12
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.16	0.06
MAGNESIUM			
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.16	0.06

Enzyme 0.05 per cent. Incubation 4.5 hours at 40 C.

bition seems to be impossible for this concentration of the enzyme as $m/4$ of the salts is almost saturation at room temperature. In this test the two salts are certainly equitoxic.

TABLE V: *Barium and strontium in contemporaneous test*

m	Control	Enzyme	Increase
BARIUM			
Water	0.13	0.27	0.14
128	0.13	0.27	0.14
64	0.13	0.24	0.11
32	0.13	0.22	0.09
16	0.13	0.20	0.07
8	0.13	0.16	0.03
STRONTIUM			
128	0.13	0.27	0.14
64	0.13	0.24	0.11
32	0.13	0.22	0.09
16	0.13	0.20	0.07
8	0.13	0.16	0.07

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In this test the two salts are certainly equitoxic. Comparison with Table IV however shows inconstancy. Thus, $m/8$ of barium in Table IV allows 0.06 as compared with 0.03 in Table V. On the other hand, in both cases, $m/128$ of barium allows uninhibited activity. Again, the figure indicating full activity in Table IV is less than that in Table V, and yet $m/8$ barium inhibits more in the latter than

in the former. It is of course simply a matter of judgment how such results are to be interpreted. Since barium and strontium are equitoxic in contemporaneous tests and since the same is true of barium and magnesium, it is reasonable to conclude that the three salts are equitoxic. This same relation was found in the case of ethyl butyrate. In this table (V) the control figure is higher than that in Table IV. There is, however, a corresponding increase in the figures in the enzyme column. Just what causes such fluctuation from day to day is difficult to tell, but there seems to be sufficient uniformity in the test as a whole. For the sake of assurance another test was made, and this gave practically identical figures with those in Table IV. The figure for $m/8$ barium shows some irregularity in this next table.

TABLE VI: *Barium and magnesium in contemporaneous test*

m	Control	Enzyme	Increase
BARIUM			
Water	0.10	0.23	0.13
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.17	0.07
16	0.10	0.16	0.06
8	0.10	0.13	0.03
MAGNESIUM			
128	0.10	0.22	0.12
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.16	0.06
8	0.10	0.16	0.06

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In the work with ethyl butyrate I found barium, strontium, and magnesium to be equitoxic with each other, but all were more toxic than either sodium, potassium, or lithium, which latter were equitoxic with each other. In the foregoing tables there are some indications that barium, strontium, and magnesium are more toxic than the sodium, potassium, lithium group. On the other hand, the differences between lithium and magnesium are not so great. Thus, $m/8$ of lithium in Table II allows 0.09, while $m/16$ of magnesium in Table IV allows 0.07. Again, $m/8$ of lithium in Table III allows 0.07. From the foregoing tables it would be impossible to say that the barium group is more toxic than the sodium group on the equinormal basis.

It may be that the zymolyte ethyl acetate tends to minimize the difference in relative toxicity as compared with the zymolyte ethyl butyrate. While this is a plausible explanation, it has little value in the absence of convincing evidence, and we cannot select the determining factor. Why the variations in the different tests should be relatively so wide is not understood, especially in view of the precautions to avoid error.[9] The following test shows that barium is more toxic than lithium under the same conditions as imposed in a contemporaneous test. This emphasizes the apparent tendency of the barium group to exceed the sodium group in toxicity, and such a conclusion is perhaps permissible in view of all the results of this work and that on ethyl butyrate, but it would certainly be difficult to say how much more toxic.

TABLE VII: *Barium and lithium in contemporaneous test*

BARIUM

m	Control	Enzyme	Increase
Water	0.10	0.20	0.10
256	0.09	0.16	0.07
128	0.09	0.15	0.06
64	0.09	0.15	0.06
32	0.10	0.13	0.03
16	0.10	0.12	0.02
8	0.10	0.10	0.00

LITHIUM

m	Control	Enzyme	Increase
64	0.10	0.20	0.10
32	0.10	0.18	0.08
16	0.10	0.17	0.07
8	0.10	0.12	0.02
4	0.10	0.11	0.01
2	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

In the preceding tests it will be remembered that the salts themselves are neutral in all the concentrations used, and that the acidity of the reagent is quite uniformly the same for all the concentrations of a given salt, and that the acidity of the control is the acidity of the enzyme solution itself, as is indicated by the figure for "Water" in the control column. It is apparent that the highest saturation is required

9 Possibly the enzyme solutions differed sufficiently to account for the discrepancies. Then too, the insolubility of the zymolyte may have been responsible to some extent.

for marked inhibition, and there is no evidence of chemical action between the enzyme substance and the toxic salt. The cause of the inhibition may be simply osmotic. In all of the remaining tests it will be noted that the toxic salt solution has more or less acidity of its own (due to hydrolytic dissociation) according to the dilution, and in several cases the control acidity is greater than the sum of the enzyme acidity and of the reagent acidity. It seems, in some cases at least, that there is chemical action between the substance of the enzyme solution and the toxic salt which results in the liberation of acid. In the higher dilutions of the toxic salt the acidity of the salt itself approaches zero, and the acidity of the control tends to be identical with that of the enzyme solution itself. Then again, at the point of total inhibition the reagent acidity and the control acidity are often equal.

TABLE VIII: *Cadmium and cobalt in contemporaneous test*

m	Reagent[10]	Control	Enzyme	Increase
CADMIUM				
Water........	....	0.10	0.22	0.12
1024.........	0.02	0.20	0.33	0.13
512.........	0.04	0.30	0.40	0.10
256.........	0.06	0.40	0.50	0.10
128.........	0.10	0.55	0.60	0.05
64.........	0.20	0.60	0.63	0.03
32.........	0.30	0.90	0.90	0.00
COBALT				
1024.........	0.03	0.20	0.33	0.13
512.........	0.06	0.33	0.43	0.10
256.........	0.08	0.43	0.52	0.09
128.........	0.10	0.70	0.77	0.07
64.........	0.15	0.90	0.92	0.02
32.........	0.30	1.00	1.00	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

All of the vials contained sediment at the close of incubation. In the unboiled *m*/1024 there was very little precipitate. Before incubation the unboiled solutions remained water clear after the mixture of enzyme and toxic salt until placed in the oven, so that the precipitation was slow.

[10] This column gives the acidity of 4^{cc} of the toxic salt solution of the concentration indicated by the corresponding figure in the column headed "*m*."

In comparison with the preceding tables there are some points of difference here. The reagent itself is acid and the unboiled preparations show a deposit of substance which tends to be zero in quantity as the dilution of the reagent increases and the acidity consequently decreases. The point of uninhibited activity lies close to that of the lowest concentration of the toxic solution having zero acidity. The acidity of the control is greater than the sum of the reagent acidity and the acidity of the enzyme solution. In this table (VIII) it will be noted that the reagent acidity for $m/32$ cadmium is 0.30, while the corresponding control figure is 0.90. A similar relation holds for cobalt. The same relation was observed for these salts in the work on ethyl butyrate. That cadmium and cobalt are more toxic than sodium, potassium, lithium, barium, strontium, and magnesium is certain. Thus it appears that the first inhibitory effect in our series, which seems more likely to be due to chemical action than to osmotic surface tension or other physical influences, is associated with substances having a natural acidity.

TABLE IX: *Copper and zinc in contemporaneous test*

COPPER

m	Reagent	Control	Enzyme	Increase
Water........	....	0.07	0.19	0.12
16384........	0.00	0.10	0.19	0.09
8192........	0.05	0.13	0.19	0.06
4096........	0.10	0.17	0.22	0.05
2048........	0.20	0.23	0.27	0.04
1024........	0.40	0.40	0.40	0.00

ZINC

m	Reagent	Control	Enzyme	Increase
16384........	0.00	0.10	0.19	0.09
8192........	0.05	0.13	0.19	0.06
4096........	0.10	0.17	0.22	0.05
2048........	0.20	0.25	0.30	0.05
1024........	0.40	0.40	0.40	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

In this test copper and zinc are certainly equitoxic. Copper is much more active in the precipitation of substance from the solution. All the unboiled solutions with zinc were free from sediment after incubation, showing that inhibition is not necessarily associated with precipitation. In the copper solutions sediment was present in all

concentrations except *m*/16384. The amount of the precipitate varies directly with the concentration of the reagent.

TABLE X: *Copper and lead in contemporaneous test*

m	Reagent	Control	Enzyme	Increase
COPPER				
Water........	....	0.10	0.20	0.10
16384........	0.00	0.10	0.20	0.10
8192........	0.05	0.15	0.20	0.05
4096........	0.10	0.18	0.22	0.04
2048........	0.20	0.23	0.26	0.03
1024........	0.40	0.40	0.40	0.00
LEAD				
16384........	0.00	0.10	0.20	0.10
8192........	0.00	0.10	0.20	0.10
4096........	0.03	0.15	0.25	0.10
2048........	0.07	0.20	0.25	0.05
1024........	0.16	0.35	0.40	0.05

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

All the vials except the *m*/16384 contained sediment at the close of incubation. The figures for copper are practically a duplicate of those in Table IX. Lead seems to be not quite so toxic as copper in

TABLE XI: *Copper, lead, and zinc in contemporaneous test*

m	Reagent	Control	Enzyme	Increase
COPPER				
4096.........	0.10	0.17	0.22	0.05
2048.........	0.20	0.23	0.26	0.03
1024.........	0.40	0.40	0.40	0.00
512.........	0.70	0.70	0.70	0.00
LEAD				
4096.........	0.03	0.16	0.22	0.06
2048.........	0.07	0.24	0.28	0.04
1024.........	0.16	0.40	0.40	0.00
512.........	0.35	0.50	0.50	0.00
ZINC				
4096.........	0.10	0.18	0.23	0.05
2048.........	0.20	0.26	0.30	0.04
1024.........	0.40	0.50	0.50	0.00
512.........	0.85	0.85	0.85	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

this test. The reagent acidity of lead is correspondingly less than that of copper, although the control figures agree fairly well with those of copper. The point of total inhibition for lead is not determined in this test, so it is not apparent whether the acidity of the reagent and control is identical at that point as was found to be the case with copper and zinc.

In this test lead proves to be equitoxic with copper and zinc. The figures for copper agree closely with those preceding. The figures for zinc agree fairly well, the important difference being that at the point of total inhibition the reagent acidity and control acidity are not identical for the *m*/1024 concentration, although at *m*/512 the coincidence may be noted. The following test is sufficient confirmation of the relation here found.

TABLE XII

COPPER

m	Control	Fnzyme	Increase
4096..........	0.13	0.20	0.07
2048..........	0.20	0.23	0.03
1024..........	0.40	0.40	0.00
512..........	0.70	0.70	0.00
		LEAD	
4096..........	0.15	0.19	0.04
2048..........	0.20	0.23	0.03
1024..........	0.30	0.30	0.00
512..........	0.50	0.50	0.00
		ZINC	
4096..........	0.15	0.20	0.05
2048..........	0.23	0.27	0.04
1024..........	0.45	0.45	0.00
512..........	0.85	0.85	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

All the unboiled zinc solutions were free from sediment. The copper and the lead solutions all contained sediment, though the *m*/4096 of each contained only the smallest traces. While total inhibition may apparently occur in the absence of any precipitation, there is always (with the exception of cadmium and cobalt in Table VIII) some inhibition accompanying precipitation.

TABLE XIII: *Mercury and silver in contemporaneous test*

MERCURY

m	Reagent	Control	Enzyme	Increase
Water........	....	0.10	0.18	0.08
65536........	0.00	0.10	0.15	0.05
32768........	0.00	0.10	0.15	0.05
16384........	0.00	0.10	0.12	0.02
8192........	0.05	0.10	0.10	0.00
4096........	0.10	0.13	0.13	0.00
SILVER				
32768........	0.00	0.10	0.15	0.05
16384........	0.00	0.10	0.13	0.03
8192........	0.00	0.10	0.13	0.03
4096........	0.00	0.10	0.12	0.02
2048........	0.00	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

In this test mercury is more toxic than silver, even on the equinormal basis, though the difference here is not nearly so great as was found with ethyl butyrate. Of course the greater concentration of the enzyme used here introduces a factor in addition to that of the different zymolyte. Mercury in comparison with silver is like copper in comparison with zinc in regard to precipitating power. The point of total inhibition is close to that of the first appearance of a precipitate,

TABLE XIV

MERCURY

m	Control	Enzyme	Increase
131072........	0.10	0.15	0.05
65536........	0.10	0.15	0.05
32768........	0.10	0.13	0.03
16384........	0.10	0.10	0.00
8192........	0.10	0.10	0.00
4096........	0.13	0.13	0.00
SILVER			
131072........	0.10	0.13	0.03
65536........	0.10	0.13	0.03
32768........	0.10	0.13	0.03
16384........	0.10	0.12	0.02
8192........	0.10	0.12	0.02
4096........	0.10	0.10	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40° C.

but with silver total inhibition may occur in the absence of any precipitation. The following test reinforces the result of this one.

In Table XIV the reagent acidity is omitted, as it may easily be seen in Table XIII and most of the values are zero anyhow. The relative toxicity is the same, athough the exact point of total inhibition is different. In the case of silver there was no deposit in any of the unboiled solutions, while with mercury there was deposit in *m*/16384 and in higher concentrations, while greater dilutions were free from deposit. To determine at what point precipitation first appears in the case of silver the following test was made.

TABLE XV: *Silver in solitary test*

SILVER

m	Control	Enzyme	Increase
Water	0.10	0.15	0.05
262144	0.10	0.15	0.05
131072	0.10	0.15	0.05
65536	0.10	0.13	0.03
32768	0.10	0.13	0.03
16384	0.10	0.12	0.02
8192	0.10	0.12	0.02
4096	0.10	0.10	0.00
2048	0.10	0.10	0.00
1024	0.15	0.15	0.00

Enzyme 0.05 per cent. Incubation 5 hours at 40 C.

The lowest concentration of the reagent to show any natural acidity was the *m*/1024, and greater dilutions were neutral to the indicator used. The only vials of the unboiled series to show a deposit after incubation were the *m*/1024.

A comparison of the results of this work on the acetate with those obtained in the work on ethyl butyrate shows that the same general relation holds for both. The metals fall into groups according to toxicity. The groups are not so clearly separated in the case of the acetate, but still sufficiently distinct to confirm the conclusion drawn in regard to the lipolysis of ethyl butyrate.

In the work on the ethyl butyrate it was found that the relative toxicity changes according to the concentration of the enzyme—a result not in accord with the conclusion of CALDWELL (*l. c.*). The following tests show that concentration of the enzyme is a factor in relative toxicity, and adds emphasis to my result with ethyl butyrate.

TABLE XVI

RELATIVE TOXICITY WITH VARYING CONCENTRATIONS OF THE ENZYME

Copper and zinc in contemporaneous test

m	Control	Enzyme	Increase
COPPER			
2048	0.27	0.32	0.05
1024	0.42	0.46	0.04
512	0.77	0.80	0.03
256	1.50	1.50	0.00
128	2.90	2.90	0.00
ZINC			
2048	0.30	0.38	0.08
1024	0.47	0.53	0.06
512	0.90	0.96	0.06
256	1.73	1.77	0.04
128	3.30	3.30	0.00

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

In Tables IX, XI, and XII, copper and zinc have the same point of total inhibition, namely *m*/1024. In those tests the concentration of the enzyme was 0.05 per cent. Here when the concentration of the enzyme is doubled the relative toxicity is changed. The point of total

TABLE XVII

m	Control	Enzyme	Increase
COPPER			
2048	0.33	0.47	0.14
1024	0.50	0.55	0.05
512	0.80	0.85	0.05
256	1950	1.50	0.00
128	2.90	2.90	0.00
ZINC			
2048	0.32	0.47	0.15
1024	0.50	0.65	0.15
512	0.87	1.02	0.15
256	1.70	1.80	0.10
128	3.40	3.50	0.10

Enzyme 0.15 per cent. Incubation 5 hours at 40 C.

inhibition for copper is *m*/256 and for zinc *m*/128. In Table XVII where the concentration of the enzyme is still higher, the difference in toxicity is more pronounced.

In the work on ethyl butyrate, cadmium, cobalt, and zinc were

found to become equitoxic with increasing concentration of the enzyme. Here the relative toxicity of zinc as compared with that of copper decreases with increasing concentration of the enzyme. In both cases the tendency of zinc to lose in relative toxicity with increasing concentration of the enzyme is apparent.

TABLE XVIII: *Mercury and silver in contemporaneous test*

m	Control	Enzyme	Increase
MERCURY			
8192..........	0.20	0.25	0.05
4096..........	0.25	0.25	0.00
2048..........	0.33	0.33	0.00
1024..........	0.35	0.35	0.00
512..........	0.53	0.53	0.00
SILVER			
8192..........	0.20	0.25	0.05
4096..........	0.20	0.24	0.04
2048..........	0.20	0.23	0.03
1024..........	0.26	0.26	0.00
512..........	0.33	0.33	0.00

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

Here the point of total inhibition for copper is the same as with the lower concentration of the enzyme in the preceding table, but the zinc

TABLE XIX

m	Control	Enzyme	Increase
MERCURY			
8192..........	0.25	0.40	0.15
4096..........	0.25	0.35	0.10
2048..........	0.35	0.40	0.05
1024..........	0.50	0.55	0.05
512..........	0.85	0.88	0.03
SILVER			
8192..........	0.25	0.35	0.10
4096..........	0.25	0.35	0.10
2048..........	0.25	0.32	0.07
1024..........	0.28	0.33	0.05
512..........	0.35	0.40	0.05

Enzyme 0.15 per cent. Incubation 5 hours at 40° C.

fails to inhibit totally and shows plainly that it loses toxicity faster than copper as the concentration of the enzyme increases.

In Tables XIII and XIV mercury is four times more toxic than

silver on the equimolecular basis. The same relative toxicity is maintained here as in those tables, although greater concentration of both salts is required for total inhibition.

Here the two salts are practically equitoxic, so that in some cases, with increasing concentration of the enzyme, salts, which are equitoxic become unequal in toxicity, while others that are not equitoxic under certain conditions become so under other conditions. Perhaps the only conclusion that can be safely drawn from this group of tests is that the concentration of the enzyme influences relative toxicity, and that a relation which holds for one concentration of the enzyme may not hold for another four or five times greater.

RELATIVE TOXICITY OF EQUICATIONIC SOLUTIONS

By calculation[11] it may be determined that copper and lead of *m*/2048 concentration are equicationic, and by examination of my results it is seen that those solutions are about equitoxic. On the other hand, *m*/1971 of barium is equicationic with copper *m*/2048,

TABLE XX: ETHYL BUTYRATE

Barium, lead, copper, and potassium in contemporaneous test

m	Control	Enzyme	Increase
BARIUM			
Water.........	0.00	0.20	0.20
2048..........	0.00	0.20	0.20
1024..........	0.00	0.20	0.20
POTASSIUM			
2048..........	0.00	0.20	0.20
1024..........	0.00	0.20	0.20
LEAD			
2048..........	0.10	0.30	0.20
1024..........	0.20	0.30	0.10
COPPER			
2048..........	0.15	0.30	0.15
1024..........	0.20	0.30	0.10

Enzyme .0125 per cent. Incubation 4 hours at 35° C.

[11] All of the equicationic calculations were made for me by Dr. WM. N. BERG of the Laboratory of Biological Chemistry of Columbia University.

but reference to the tables shows that such a concentration of barium is entirely without inhibitory effect upon the concentration of the enzyme tried. So by calculation it is seen that *m*/2030 of potassium is equicationic with copper *m*/2048, but reference to the table shows that potassium of such concentration is not toxic. In order to leave no doubt that cation concentration is not a determining factor in toxicity to the extent that equicationic solutions are equitoxic, the following tests were made.

According to calculation, the following solutions are equicationic: copper and lead *m*/2048, potassium *m*/2030, barium *m*/1971. Instead of making the exact concentrations for barium and potassium, it was assumed that if *m*/1024 and *m*/2048 do not inhibit, intermediate concentrations would not. Examination of the results shows that there is no basis for regarding equicationic solutions as even probably equitoxic. Neither of the concentrations of barium tried nor of potassium are inhibiting, while both of copper and one of lead are. By calculation it is easy to see that there is no fixed relation apparent between equitoxicity and equicationic concentration. The exceptions may be multiplied. Thus in the butyrate tests in my other paper, copper *m*/512 and lead *m*/512 are equicationic, equidissociated, and equitoxic. On the other hand, barium *m*/8 and copper *m*/512 are equitoxic, but the relative number of cations per unit volume of those elements in those concentrations is 38 to 1. That is, in unit volume of barium *m*/8 there are 38 cations as compared with 1 cation in unit volume of copper *m*/512. The test shown in Table XXI with ethyl acetate shows that equicationic solutions may totally inhibit or may not inhibit at all according to the salts compared.

According to the calculation from the conductivity tables, the following nitrates have a cation concentration of *m*/80.7: barium *m*/64, copper *m*/70, lead *m*/64, and potassium *m*/76. Reference to the above test shows that all of those concentrations are included within the limits of the test, and yet copper and lead totally inhibit, while barium and potassium do not inhibit at all.

DISCUSSION

The attempt to correlate physiological action with certain physical or chemical properties either of the ions or of the atoms has thus far

been unsuccessful.[12] In view of the really large attention that has been devoted to this subject, it seems surprising that no generalization has been satisfactorily established (BERG and GIES, *l. c.*). The affinity of ions for their charges is such a fundamental property that we can hardly conceive of a chemical reaction involving solution in which this affinity or solution-tension, as some have called it, does not play a part. On the other hand, there is abundant evidence offered by my

TABLE XXI: ETHYL ACETATE

Barium, lead, copper, and potassium in contemporaneous test

m	Control	Enzyme	Increase
COPPER			
Water..........	0.12	0.27	0.15
128...........	2.90	2.90	0.00
64...........	5.70	5.70	0.00
LEAD			
128...........	2.10	2.10	0.00
64...........	4.20	4.20	0.00
BARIUM			
128...........	0.12	0.27	0.15
64...........	0.12	0.27	0.15
POTASSIUM			
128...........	0.12	0.27	0.15
64...........	0.12	0.27	0.15

Enzyme 0.10 per cent. Incubation 5 hours at 40 C.

experiments to show that there is no fixed relation between toxicity and solution-tension to the extent that the toxicity of substances can be exactly estimated from their solution-tension (POND, *l. c.* 274). There is difficulty even in agreeing upon a standard of toxicity. The minimum lethal dose measures the fatal toxicity, but is it a standard of toxicity? Does total inhibition measure the toxicity in my own experiments? In Table XIII we see that $m/16384$ and $m/32768$ of both mercury and silver are inhibiting and equally inhibiting, but for

[12] The following paper offers a recent and thorough digest of the more important literature of this subject: BERG, WM. N., The relation between the physiological action of ions and their physico-chemical properties. New York Medical Journal for July 20 and July 27, 1907. pp. 42.

total inhibition four times greater equimolecular concentration of silver is required than of mercury. (See also POND, *l. c.* 268.)

A critical examination of my own results and those of others fails to reveal any general relation without exceptions, or any property or factor that can be regarded as exclusively fundamental and as solely determining the relative toxicity of a wide series of salts.

Each of the salts I have tested is a nitrate except one, the chlorid of mercury. The members of the series fall into two groups, the neutral salts which do not hydrolytically dissociate in aqueous solution, and the hydrolytes which do so dissociate and whose solution is therefore acid or alkaline according to whether the H or OH ion is predominant. All of the hydrolytes of my series are acid, but as the dilution increases the acidity decreases to zero as here tested.

This difference between the neutral salts and the hydrolytes shows very clearly in the acidity of the controls. The controls of the neutral salts have practically the same acidity as the enzyme solution itself, that is, there is no evidence of any alteration of the acidity of the boiled enzyme solution as a consequence of the mixture with the neutral salt solution. Thus in Tables I–VII inclusive the acidity of the control is about the same for each concentration of the salt. This acidity is close to if not identical with the acidity of the plain boiled enzyme solution as indicated by the figures corresponding to "Water."

In the other tables, however, where the salts have an acid reaction in consequence of their hydrolytic dissociation, the acidity of the control and of the reagent varies considerably (POND, *l. c.* 283). Thus in Table VIII the acidity of the plain control without salt present is 0.10. The acidity of $m/1024$ cadmium is 0.02, but when the control for $m/1024$ is observed, the figure is 0.20 or considerably more than the sum of reagent and enzyme. In regard to this behavior there is much variation among the hydrolytes. Thus at the point of total inhibition the reagent acidity and control acidity may be identical, as with copper and zinc in Table IX, or considerably different, as in Table VIII with cadmium. It is difficult to interpret this with assurance.

It is notable that the neutral salts are not of equal toxicity either in the saponification of the acetate or of the butyrate (POND, *l. c.* 265, 266). Thus in Table VII barium is more toxic than lithium, so that we cannot correlate the degree of toxicity with the degree of

hydrolytic dissociation in any close way, though it is true that all of the hydrolytes in the tests I have made are more toxic than any of the neutral salts.

The fact that the hydrolytes themselves are potent in saponification has long been known, in fact the degree of hydrolysis has been measured by the rate of the saponification of esters.[13] Such salts have also been found to be potent in the inversion of sugars.[14] It was supposed that the inverting or saponifying power is associated with hydrolytic dissociation, the H or the OH ions being the active agents. LEY[15] (p. 214), however, found that KCl can invert sugar, so that other ions than those resulting from hydrolytic dissociation are potent. Thus it seems that exceptions arise to intercept the formulation of any generalization.

Significant also is the fact that dilutions of silver and mercury so great that the reaction is neutral as tested are capable of causing total inhibition. On the other hand, solutions of the neutral salts which are comparatively very concentrated are not inhibiting. Thus in Table XIII, *m*/2048 of silver is totally inhibiting but not measurably acid. In some cases we find that solutions of equal acidity are also equitoxic (Tables VIII and IX especially). This relation, however, fails in some cases (Tables X and XI). Moreover, partial or total inhibition may occur with the hydrolytes at dilutions too great to show acidity as here tested. Thus copper and zinc in Table IX show partial inhibition at *m*/16384, which is neutral, and mercury at *m*/16384 in Table XIII shows almost total inhibition without acidity, while silver in Table XIII shows total inhibition in a neutral concentration of the salt.

The relative toxicity changes as the concentration of the enzyme changes, provided the difference is enough. Thus in Tables IX, XI, and XII copper and zinc are equitoxic, but in Table XVI they are less equal, and in Table XVII still less so with the increasing concentration of the enzyme. Similar variations were found in the tests with ethyl butyrate (POND, *l. c.* 276).

13 SHIELDS, JOHN, Ueber Hydrolysis in wässerigen Salzlösungen. Zeitsch. physikalische Chem. **12**:167–187. 1893.

14 LONG, J. H., On the inversion of sugar by salts. Jour. Amer. Chem. Soc. **18**:120–130. 1896.

15 LEY, H., Studien über die hydrolytische Dissociation der Salzlösungen. Zeitsch. physikalische Chem. **30**:193–257. 1899.

Just to what extent precipitation during incubation is a factor in inhibition is difficult to say. The results show that precipitation occurs in concentrations which cause total and often only partial inhibition. However, with zinc (Tables IX and XII) and silver (Tables XIV and XV) we find total inhibition without precipitation.

The enzyme itself, that is, the substance exhibiting zymolytic power, is probably a reversible colloid, and since it was not precipitated by the neutral salts used, it may be assumed to have gone into colloidal solution rather than into suspension. Perhaps it also is a hydrolyte liberating both H and OH ions. If this is the case, its saponifying power may be associated with its hydrolytic dissociation, just as has been that of the salt hydrolytes. The toxicity of the salt might even be referred to the possibility that the excess of H or of OH ions resulting from the hydrolysis of the salt prevented the hydrolytic dissociation of the enzyme substance and therefore caused the inhibition[16] (p. 214). If so, to what is the toxicity of the neutral salts to be referred? Also, why are the very highly diluted and therefore neutral solutions of silver and mercury capable of causing total inhibition? May not the chemical nature of the atom be a factor? Since convincing evidence is not available for a negative answer, and since theoretical argument based upon the negative assumption leads to embarrassing conclusions,[17] it is obvious that in any effort to discover final causes this possibility should not be ignored.

The tests with equicationic solutions show the futility of any attempt at generalization in that direction and at every hand exceptions seem to arise.

CONCLUSION

In the zymolytic saponification of ethyl acetate as in that of ethyl butyrate, the toxicity of the salts tested does not under the conditions specified vary inversely with the decomposition tension of those salts.

The concentration of the enzyme is a factor in relative toxicity in some cases.

16 BREDIG, G., Beiträge zur Stoichiometrie der Ionenbeweglichkeit. Zeitsch. physikalische Chem. **13**: 191–288. 1894.

17 BERG, *l. c.* pp. 15–20.

NOTE.—In referring to POND (*l. c.*), note the following errata: p. 274, bottom line, "Zinc and cadmium" should read Zinc and barium; p. 283, line 13 from top, "Table XIII" should read Table XIV.